Media and Agar

Aim

Prepare LB and SOC media and agar, and 5x KCM.

Procedure

LB Medium

1. To make 1L LB medium, mix the following:

Component	Amount [g]
Yeast extract	5
NaCl	10
Tryptone	10
dH_2O	950 ml

2. Adjust the PH to 7.0 with 1 M NaOH, then autoclave.
3. Autoclave your prepared medium.

LB Agar

1. Before autoclaving, add 15 g/L agar to the LB media.
2. Cool to approximately 55 °C add antibiotics.
3. Pour into the plate (10 ml/plate), stock plates and let harden.
4. Invert and store at 4 °C.

SOC Medium

1. To make 1L SOC medium, mix the following:

Component	Amount [g]
Tryptone	20
Yeast extract	5
NaCl	0.585
KCl	0.186
$MgSO_4$	1.2
$MgCl_2$	0.95 (or 2.02 g $MgCl_2{\cdot}6H_2O$)
dH_2O	950 ml

2. Adjust to pH 7.5 prior to use. This requires approximately 25 ml of 1 M NaOH per liter.
3. Autoclave. After cooling medium to less than 50 °C, add 20 ml filter sterilized 20 % glucose solution (4 g glucose into 20 ml).

5x KCM

1. To make 5x KCM, mix the following:

Component	Concentration [M]	Example amount [g]
KCl	0.5	37.25
$CaCl_2$	0.15	16.65
$MgCl_2$	0.25	50.5 ($MgCl_2 \cdot 6H_2O$)
dH_2O	Fill upp to total volume	Fill up to 1 L

2. Divide into 1 ml aliquots

1xTAE Buffer

1. To make 50xTAE Buffer, mix the following:

Component	Concentration [M]	Example amount
Tris base	-	242 g (in water)
Acetic acid	-	57.1 mL
EDTA	500 mM (pH 8.0)	100 mL
dH_2O	Fill upp to total volume	Fill up to 1 L

2. If a 1xTAE Buffer is wanted, the solution can be diluted 50:1 with dH_2O. This 1X solution will then contain 40mM Tris, 20mM acetic acid, and 1mM EDTA.

1 % Agarose mixture with GelRed dye for gel electrophoresis

1. To make a 1% Agarose mixture, mix the following:

Table 1: Table of Agarose components

Component	Example amount
Agarose	1g
1xTAE Buffer	100mL

2. After mixing the agarose and buffer in a flask according to 1, put it in the microwave and heat it for as long is takes to completely dissolve the agarose. Be careful not to burn yourself, the liquid will become very hot. Swirl the mixture to dissolve the agarose more efficiently.
3. Add 10 μl (1 % concentration) GelRed dye to the flask and mix it carefully.
4. Pour the agarose mixture into your casting tray and let it harden before loading your sample and running the gel electrophoresis.

Sources

Recipes are modified from Cold Spring Harbor Protocols, 2016.